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Published in:
Building and Environment

DOI (link to publication from Publisher):
10.1016/j.buildenv.2015.03.031

Publication date:
2015

Document Version
Early version, also known as pre-print

Building and Environment xxx (2015) 1–8

Contents lists available at ScienceDirect

Building and Environment

journal homepage: www.elsevier.com/locate/buildenv

Impact of dwelling characteristics on concentrations of bacteria, fungi, endotoxin and total inflammatory potential in settled dust

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ARTICLE INFO

Article history:
Received 17 December 2014
Received in revised form
25 March 2015
Accepted 30 March 2015
Available online xxx

Keywords:
Fungi
Endotoxin
Dust collector
Inflammatory potential
Settled dust
Dwelling characteristics

ABSTRACT

Indoor air in homes contains a variety of organic agents such as bacteria, endotoxin and fungi. Epidemiological studies have shown links between these components and respiratory problems and the development of allergies.

Twenty-eight dwellings located in the Greater Copenhagen area in Denmark were investigated in this study. Temperature, relative humidity and air exchange rate were measured. Dwelling characteristics including floor area, volume of the living room, floor material, year of construction of buildings and floor level were collected. The microbial exposure was measured by quantifying fungi, bacteria and endotoxin concentration in airborne dust collected by Electrostatic Dust fall Collectors (EDCs). The Total Inflammatory Potential (TIP) of the dust was also measured.

Significantly higher concentrations of fungi were found in dwellings with high relative humidity ($p = 0.03$), larger room volume ($p = 0.03$) and in dwellings located on the second floor or higher ($p = 0.02$). Small floor area per person and low air exchange rate were significantly associated with increased concentrations of bacteria (both $p < 0.01$). Spring season ($p = 0.01$), buildings constructed before the 20th century ($p = 0.09$) and wooden floor ($p = 0.03$) were associated with high TIP.

In conclusion, people living in smaller dwellings or in dwellings on upper floors are at higher risk of microbial exposure. While TIP was affected by some dwelling characteristics, it was mainly influenced by season.

1. Introduction

Varieties of fungal and bacterial genera are commonly found indoors in the form of airborne particles called “bioaerosol”. Along with other pollutants, fungi and bacteria in settled dust can become airborne [13]. Airborne microorganisms have a tendency to aggregate into particles of different sizes depending on source, species, relative humidity and the mechanism of aerosolization [26,36]. Several studies have indicated that the presence of fungi, bacteria and endotoxin in the indoor environment is associated with serious inflammation-related health risks such as asthma, allergies and respiratory discomfort [18,32,58]. Furthermore, several studies indicate that dose response relationship between exposure to microorganisms and the development of asthma exacerbate the symptoms [16,19,28,31,41,42].

The evaluation of TIP is based on the differentiated HL-60 cell-line, which, upon exposure to microbial compounds, reacts by producing reactive oxygen species (ROS). Human airways and lung alveoli contain small number of granulocytes. In the human body the pulmonary vasculature is the largest reservoir of granulocytes. The pulmonary vasculature can distribute the granulocytes rapidly and thus respond against pathogens [53]. Although the isolated

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http://dx.doi.org/10.1016/j.buildenv.2015.03.031
0360-1323/

granulocytes are short-lived [60], the production of ROS of granulocytes can represent a readout for microbial exposure. ROS can be quantified by a luminol dependent chemiluminometric assay.

Fungi and bacteria, as well as endotoxin, are present in the outer cell wall of gram-negative bacteria that naturally occur outdoors. Levels of outdoor fungi are affected by local climate conditions, such as rainfall and temperature [12]. Outdoor concentrations of bacteria and fungi contribute to the indoor levels via infiltration through the building envelope [21]. However, studies comparing the indoor and outdoor levels of bacteria often find the indoor/outdoor ratios are above 1 [2,6,21,34,52], showing the importance of indoor sources. Several studies have suggested that in environments like homes [35], university buildings [37], schools [6] and day-care centers [4], the main source of airborne bacteria indoors are humans and human activities. Bacterial exposure levels in homes are influenced by many factors, including, for example, relative humidity of indoor air and seasons during the year [22,44]. Moisture has a great influence on fungal growth and bacterial viability [59]. The temperature, ventilation rate and construction details are often reflected in the year of construction which, have been shown to influence the moisture level in dwellings [9,27,45].

Measurement of fungi, bacteria, endotoxin and assessment of TIP can be costly. It is not clear whether building characteristics can be used as a proxy for these microbial agents. Moisture-related problems in buildings have been interlinked to respiratory problems [24,27,29]. Although the relationship between moisture in homes and microbial exposure is well studied, the relationship between building characteristics is not yet fully understood. Improved understanding would be valuable in assessing the extent to which the building characteristics can serve as a surrogate for measurements of microbial exposure.

The objective of this study was to i) investigate possible associations between building characteristics and concentrations of fungi and bacteria and endotoxin in tested dwellings, and ii) investigate associations between building characteristics and total inflammatory potential of settled dust.

2. Methodologies

Twenty-eight dwellings in the Greater Copenhagen area were investigated in this study. Characteristics of the dwellings are given in Table 1. All dwellings were situated within a radius of 1600 m from major roads (with more than 70,000 vehicles per 24 h) and were not equipped with mechanical supply ventilation. Aside from traffic, no other significant source of pollution was present nearby. Neither visible mold nor water damage was observed in the dwellings throughout the experimental period. A detailed description of the study design and investigated dwellings is presented in Ref. [55].

The residents were elderly couples (in 22 dwellings) and individuals (6 dwellings). They were non-smokers. They spent 83% (median) of their time at home during the study period. All participants were above 51 years of age and had been living in their dwellings for at least four years. One couple was later excluded as the study was terminated in their dwelling due to complaints of noise, leaving 48 participants living in 27 dwellings for analysis.

The measurements included number concentrations of ultrafine particles (UFP), $PM_{2.5}$, air exchange rate (AER), temperature and indoor air humidity, and the investigated bioaerosols: bacteria, fungi and endotoxin, as well as TIP. The measurements in each apartment lasted for 14 days and all measurements were completed within a seven-month period starting November 2010.

Table 1
Building characteristics and settings in the dwellings.

ID	T[a] (°C)	RH[a] (%)	Area p. person (m^2/person)	Living room volume (m^3)	Floor level	Flooring material[b]	Pets	Year of construction	AER (h^{-1})	Exp. period[c]	$PM_{2.5}$[d] ($\mu g\ m^{-3}$)	UFP[e] (# cm^{-3})
1	20.30	29.70	10.9	68	1st	W	No	1903	0.587	W	6.76	7079
2	20.90	26.80	9.8	51	G	W	No	1891	0.803	W	6.84	17,620
4	21.30	40.40	5.2	54	G	W	No	1884	0.198	W	–[f]	27,488
5	21.50	33.60	15.2	76	G	P	No	2004	0.250	W	8.11	10,945
6	21.30	37.00	13.8	76	3rd	P	Yes	1939	0.146	W	8.99	6327
7	21.10	44.50	10.5	53	4th	C	No	1954	0.251	W	9.45	–[f]
8	23.00	24.70	9.3	46	4th	W	Yes	2003	1.266	W	8.38	3706
9	21.60	29.20	15.8	81	4th	W	No	2005	0.806	W	6.56	5255
10	22.60	32.30	7.6	40	1st	W	No	1883	0.568	W	12.27	7669
11	22.90	29.60	12.3	65	2nd	W	No	1892	0.295	W	6.19	16,946
12	23.83	24.85	11.9	60	2nd	P	Yes	1979	0.486	S	9.39	16,286
13	23.46	23.15	18.0	104	4th	P	Yes	1896	0.878	S	13.32	8893
14	21.10	30.40	17.5	83	5th	W	Yes	1927	0.506	S	12.71	4023
15	22.28	25.08	7.6	46	G	W	No	1901	0.577	S	14.2	13,677
16	22.38	24.90	27.5	149	4th	W	No	1924	0.549	S	2.77	16,053
17	23.37	27.19	16.2	104	G	P	No	1902	–[f]	S	7.46	4036
18	23.25	16.67	39.2	100	5th	C	No	1974	–[f]	S	5.55	–[f]
19	20.96	35.71	9.6	55	3rd	W	No	1895	0.451	S	11.12	94,783
20	21.92	27.17	18.0	112	1st	C	No	1893	0.366	S	–[f]	3090
21	21.99	27.75	28.5	162	4th	W	No	2001	0.514	S	5.46	7155
22	21.60	36.95	6.9	35	3rd	W	No	1956	–[f]	S	6.2	8662
23	21.72	32.69	17.8	42	2nd	W	No	1797	0.654	S	7.62	13,692
24	22.97	34.78	27.0	71	2nd	W	No	1800	0.396	S	3.41	4449
25	23.20	37.30	19.1	124	1st	C	No	1902	0.317	S	9.61	3435
26	22.86	43.18	19.1	106	2nd	W	No	1921	0.289	S	13.95	5506
27	23.36	34.77	19.1	52	3rd	W	No	1904	0.197	S	2.32	–[f]
28	21.99	38.30	21.2	52	2nd	W	Yes	1995	0.639	S	9.33	–[f]

[a] Average value over the whole two-week exposure period.
[b] Floor material in living room: W – wooden flooring, cork or linoleum; P – parquets; C – carpet.
[c] Experimental period: W – Winter (November–February), S – Spring (February–May).
[d] Average value over two-week exposure period.
[e] Average value over 24-h measurement. Measurement in dwelling no 3. was terminated and excluded from analyses.
[f] Missing data due to failure of measuring device.

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The UFP concentrations (# cm^{-3}) were measured by means of continuous monitoring using NanoTracer PNT 1000 (Philips Electronics N.V., Netherlands) placed in the living room. The NanoTracer counted particles in the range from 10 nm up to 300 nm. The UFP are defined as particles smaller than 100 nm, thus also slightly larger particles than in the usual UFP cut-off of 100 nm were included. The UFP measurements consisted of representative 24-h period. The $PM_{2.5}$ concentrations were measured using a cyclone sampling head GK 2.05-KTL (BGI Incorporated, USA) powered by BGI 400 sampling pumps (BGI Incorporated, USA) with a constant sampling rate of 4 l min^{-1}. The pumps collected particles continuously over the entire study period; however, the filters were changed after seven days. Concentrations of $PM_{2.5}$ were calculated as mg m^{-3}. Details of the procedure are given in Ref. [55].

Temperature (°C) and relative humidity (%, RH) were measured using TinyTags (Gemini Data Loggers, UK), which were placed in the living rooms. The TinyTags were set to measure in a continuous mode giving the average in ten-minute intervals. Perfluorocarbon tracer-gas method (PFT) was used to measure the air exchange rates (AER, h^{-1}) in the dwellings. The dwellings were treated as single zones. Details of the PFT technique including analysis of tracer gases have been described previously [7]. The uncertainty of single-zone tracer-gas measurements is below 20%.

Dwelling characteristics collected by researchers included floor area, volume of the living room, flooring material, year of construction, floor level of the dwelling and the presence of pets.

Electrostatic Dust fall Collectors (EDCs) were used to collect settled dust. The EDC consists of four polypropylene, electrostatic fleece cloths (ZEEMAN Alphen, Holland) each with a surface area of 0.02 m^2 (0.19 × 0.11 m). EDCs have proven effective for measuring endotoxin units (EU m^{-2} day^{-1}) as well as for and fungal colony forming units (CFU m^{-2} day^{-1}) and bacteria (CFU m^{-2} day^{-1}) [21,40]. In 20 dwellings one EDC was placed at the top of bookshelves in the living room for a period of 14 days. Due to space constraints, in seven dwellings the EDC was placed not on the top of the bookshelf but between bookshelves. The average height of EDC locations was 1.83 ± 0.23 m. Fungi, endotoxin and bacteria present in the dust on the EDCs were extracted and quantified as described elsewhere [35].

To measure the TIP of indoor dust different cell based *in vitro* assays have been used based on the Human Promyelocytic Leukaemia cell line (HL-60) [21] a human macrophage cell line [33], a lung epithelial cell line (A549) [3,33,51]. The detailed procedure and evaluation of TIP (TIP m^{-2} day^{-1}) of settled dust is described in Refs. [35,57]. The Electrostatic Dust fall Collector (EDC) has been used for sampling sedimenting microorganisms and other particles including endotoxin in the indoor environment [35]. The EDC was chosen for this study because of its time integrating sampling ability, which is important given that indoor exposure varies during a day due to different activities and occupancy levels [35] and the opening of windows [34]. The ability of collected dust samples to induce ROS production was evaluated by quantifying the TIP of settled dust. The TIP assay is based on the Human Promyelocytic Leukaemia cell line (HL-60) differentiated to granulocytes like cells [11,15]. Reasons for the assay to be relevant for assessment of potential airway inflammation caused by indoor exposure is that neutrophilic and eosinophilic granulocytes are elevated in subjects with asthma [10,61]. Neutrophilic granulocytes can be also induced as a response to exposure to bioaerosols [30,39]. Finally, as cell activation is related to the multifactorial composition of the bioaerosol sample, it could be used as a measurement of the TIP of a given sample.

2.1. Statistical analyses

Statistical analyses were conducted using SPSS® IBM® (Ver. 20.0.0, 2011). A General linear model (GLM) was used to evaluate the influence of measured and observed dwelling characteristics on concentrations of fungi, bacteria and endotoxin. The tested explanatory variables included: air temperature (°C), indoor air relative humidity (%), area per person (m^2 $person^{-1}$), volume of the living room (m^3), floor level (ground floor to1st floor; 2nd floor and higher), floor material (carpet; wood or linoleum), year of construction (built before 1900; between 1901 and 1960; 1961 or later), presence of pets (present; not present) and air exchange rate (0–0.5; above 0.5 h^{-1}). In analyses of the influence of building characteristics on TIP, we included concentrations of $PM_{2.5}$ (μg m^{-3}) and particle number concentration of UFP (# cm^{-3}). For descriptive purposes, the influence of the variable floor level on TIP was evaluated for each floor level individually, except the 5th floor. The 5th floor was grouped together with the 4th floor.

For all statistical analyses, the following approaches were used: i) univariate regression analyses, where each explanatory variable was included individually in the model; and ii) a multiple regression approach, including all explanatory variables with mutual adjustment. The influence of the explanatory variables on microbial exposure were expressed as a percentage change in concentrations of fungi, bacteria, endotoxin and TIP according to each given increment in the linear determinant variable or class variable. Analysed data for fungi, bacteria and endotoxin was not normally distributed. However, the data was normally distributed after natural logarithmic transformation and the logarithmically transformed data was used for the statistical analysis.

Pearson correlation coefficients were calculated to estimate correlation between the season (winter or spring) and concentrations of bacteria, TIP and fungi and between $PM_{2.5}$ and fungal concentrations.

3. Results and discussion

In the present study we evaluated possible associations between dwelling characteristic and levels of fungi, bacteria and endotoxin in settled dust, as well as TIP, in 27 Danish dwellings. The characteristics of the dwellings are given in Table 1.

3.1. Concentrations of fungi

The fungal concentrations ranged from 127.6 to 6515 CFU m^{-2} day^{-1} with a geometric mean of 1442 CFU m^{-2} day^{-1}. Table 2 shows associations between nine dwelling characteristics and fungal concentrations. In the multivariate analysis, the amount of fungi was significantly higher in apartments with high indoor air relative humidity ($p = 0.03$), room volume ($p = 0.03$) and on higher floor levels ($p = 0.02$). The squared coefficient of multiple correlation for multivariate association was relatively low ($R^2 = 0.48$, Table 2) in this study, which means that our model does not fully explain the variation in fungal concentrations. This can either hide some important associations or result in false-significant results, thus the data should be interpreted with caution.

In this study, larger rooms were significantly associated with higher concentrations of fungi ($p = 0.05$ and $p = 0.03$ for univariate and multivariate association, respectively). Significantly higher fungi concentrations were also found in dwellings with larger area per person ($p = 0.05$). However, this was only true in the univariate model. In the multivariate model, this association changed direction and lost significance (Table 2). This could be explained as a result of interference with variable RH in the multivariate analysis. Pearson correlation between RH and area per person showed an

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Table 2
Concentrations of fungi measured in tested dwellings.

	Univariate association		Multivariate association	
	% difference (95% CI)[a]	P-value	% difference (95% CI)[b]	P-value
Temperature (°C)	4.0 (−9.5; 17.5)	0.58	35.5 (−5.4; 46.5)	0.18
Indoor air RH (%)	4.0 (−0.3; 8.3)	0.08	9.4 (1.8; 17.1)	0.03
Area p. person (m^2/pers.)	3.7 (0.1; 7.2)	0.05	−0.9 (−7.4; 3.1)	0.10
Room volume (m^3)	0.8 (0.0; 1.6)	0.05	1.3 (0.3; 2.9)	0.03
Floor level				
>1st floor (n = 18)	42.5 (−15.9; 101)	0.24	124.1 (57.9; 190)	0.02
ground−1st floor (n = 9)	–	–	–	–
Floor material				
Carpet (n = 4)	51.7 (−26.1; 130)	0.30	43.3 (−33.7; 120)	0.28
Wood or linol. (n = 23)	–	–	–	–
Pets				
No (n = 21)	87.2 (22.3; 152)	0.06	78.6 (−10.1; 161)	0.11
Yes (n = 6)	–	–	–	–
Year of construction				
17th century − 1900 (n = 9)	−44.1 (123; 34.5)	0.15	−43.1 (−170; 34.3)	0.12
1901−1960 (n = 11)	4.5 (−71.4; 80.4)	0.91	73.3 (−49.0; 195.6)	0.32
1961−present (n = 7)	–	–	–	–
AER (h^{-1})	−78.8 (−187; 29.8)	0.1	−69.9 (−145; 15.4)	0.16

[a] Estimated change in average fungi concentrations (in CFU m^{-2} day^{-1}). For example, a change of the variable floor level 42.5 is interpreted as 42.5% increase of CFU m^{-2} day^{-1} of fungi in dwellings located above the first floor compared with group of dwellings on the ground and first floor.
[b] The model in the multivariate association explained 48% of the variance ($R^2 = 0.48$).

inverse correlation ($r = -0.348$, $p = 0.08$). Although the correlation is not significant, the variable, together with the variable area per person, showed the highest significance level among tested variables. A similar conclusion was drawn by Ref. [45], who found relative humidity to have a more significant impact on concentration of fungi as compared to the impact of the occupied area. The positive association between relative humidity and concentrations of airborne fungi aligns with the fact that high relative humidity allows fungal growth. However, the observation contrasts with the more transient observation that more spores are aerosolised from many different common indoor fungi at high air velocity if the fungal colonised area is dried out [20,23]. High relative humidity has also been shown as a factor to increase fungal concentrations in other continents and cultures [5,45]. Analysing the impact of occupants' presence [35], showed fungal levels of 8.2×10^2 CFU m^{-2} day^{-1} (average level) in absence of occupants and of 3.0×10^3 CFU m^{-2} day^{-1} (average level) when occupants were present. This difference was not significant in this study, however.

Elevated fungal concentrations measured in dwellings on higher floor levels ($p = 0.02$) contrast with results of [49], who found the highest levels of fungi on the ground floor. The difference might be explained by different building characteristics in our study compared to [49]. In Denmark, the Danish government released the "The Building Act of 1856," legislating a thinner exterior building wall and increasing floor levels of the building. The thin uninsulated building exterior walls might have increased the risk of water condensation in dwellings and thus enhanced the growth of fungi on the wall surface. It may also be speculated if the roof structures are a significant source of fungal spores. Finally, the apartments are not vertically airtight and the apartments at higher floor levels are – because of the "stack effect" – expected to have a transfer of air from apartments below to account for much of their air exchange. There may be an accumulation of mold spores in the transferred air as it moves from one floor to the next.

In our study we did not find a correlation between airborne $PM_{2.5}$ and fungal concentration ($r = -0.04$, $p = 0.83$). In previously conducted studies particle-size intervals of fungi present in indoor air have been reported mostly within the upper limit of 4.7 μm in diameter [38,47]. Another study has quantified size-resolved fungal concentrations collected under both an occupied and a vacant state in a school classroom [43]. In both states PM number was increased predominately in the 3–5 μm range. It is most likely that the upper-size limit of the PM collected in our study (i.e. 2.5 μm in diameter) was unable to include the entire size range of fungi mass.

3.2. Bacteria concentrations

The concentrations of bacteria were found to range from 102.5 to 23,923 CFU m^{-2} day^{-1} with geometric mean 2302 CFU m^{-2} day^{-1}. Uni- and multivariate associations between analysed building characteristics and bacterial levels are displayed in Table 3. The coefficient of determination is slightly higher for the multivariate analysis between bacteria concentrations and dwellings characteristics ($R^2 = 0.57$, Table 3) than fungi, but still too low to fully explain the variation in concentrations. Area per person was significantly correlated with bacteria levels, such that higher levels of bacteria were found in apartments with smaller area per person ($p < 0.01$). This is in accordance with what was earlier found by Ref. [48], who reported a significant inverse association between size of occupied area and concentration of bacteria.

In this study a statistically significant correlation was observed between low CFU of bacteria and spring season (Pearson coefficient $r = 0.38$, $p = 0.02$, data not shown). The results from earlier studies are contradictory. While higher indoor levels of bacteria were reported during spring, compared to winter in a Danish study [21], in Finland slight yet significant differences between seasons (winter and summer) have been observed, with the largest differences in winter [46].

High bacteria levels were associated with lower air change rate ($p = 0.04$ and $p < 0.01$ for univariate and multivariate association, respectively). In agreement with our findings [25,17], have reported high bacteria and bacterial endotoxins to be strongly associated with inadequate ventilation-related habits (i.e., low air exchange rate).

Low levels of bacteria were measured in dwellings located on higher floor levels in univariate analysis ($p < 0.01$), but this association lost significance in the multivariate analysis (Table 3).

Bacteria levels were not associated with concentrations of $PM_{2.5}$ mass. Similar to fungi, bacteria levels were in another study found to be correlated with larger particle size-ranges (3–5 μm in diameter in collected mass) [43].

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Table 3
Bacteria levels measured in tested dwellings.

	Univariate association		Multivariate association	
	% difference (95% CI)[a]	P-value	% difference (95% CI)[b]	P-value
Temperature (°C)	−6.1 (−21.4; 9.2)	0.45	35.4 (−3.6; 25.8)	0.17
Indoor air RH (%)	3.4 (−1.5; 8.3)	0.19	5.9 (−0.8; 9.2)	0.15
Area p. person (m^2/pers.)	−7.0 (−10.6; −3.5)	<0.01	−10.9 (−17.1; −4.6)	<0.01
Room volume (m^3)	−0.8 (−1.6; 0.0)	0.07	1.4 (−0.1; 2.7)	0.1
Floor level				
>1st floor (n = 18)	−61.8 (−124; 0.0)	<0.01	−49.3 (−119; 24.2)	0.09
ground−1st floor (n = 9)	–	–	–	–
Floor material				
Carpet (n = 4)	−48.8 (−136; 38.2)	0.14	−21.3 (−46.8; 4.1)	0.11
Wood or linol. (n = 23)	–	–	–	–
Pets				
No (n = 21)	1.5 (−74.5; 77.6)	0.97	28.1 (−58.3; 108)	0.67
Yes (n = 6)	–	–	–	–
Year of construction				
17th century−1900 (n = 9)	81.1 (−11.8; 174)	0.22	22.4 (−97.6; 64.0)	0.38
1901−1960 (n = 11)	133 (−42.8; 223)	0.07	−69.9 (−84.2; 86.5)	0.07
1961−present (n = 7)	–	–	–	–
AER (h^{-1})	−77.7 (−136; −18.9)	0.04	−86.7 (−149; −24.0)	<0.01

[a] Estimated change in average bacteria levels (CFU m^{-2} day^{-1}) according to the reference condition. For example a change of the variable floor material −48.8 is interpreted as 48.8 decrease of bacteria level in dwellings equipped with carpet in the living room compared with a group of dwellings with wood or linoleum as a floor material in the living room.
[b] The model in the multivariate association explained 57% of the variance ($R^2 = 0.57$).

3.3. Levels of endotoxin

The geometric mean concentration of endotoxin was 119 EU m^{-2} day^{-1}, ranging between 11.2 and 1077 EU m^{-2} day^{-1}. Concentrations of endotoxin were significantly negatively associated with area per person in the univariate model ($p = 0.01$, Table 4). This association lost significance in the multivariate model. However, it remained as the most important variable in the model, with $p = 0.08$. Similar to our findings, two other studies by Refs. [25,17] measured significantly higher levels of endotoxin in dwellings occupied by higher number of persons and in 10 California homes significant correlations have been found between occupancy and endotoxin exposure [14]. In another study differences in endotoxin exposures have been found when occupants were absent from their home for two weeks. During occupancy the levels of endotoxin were 650 EU m^{-2} day^{-1} while during occupant absence the levels dropped to 39 EU m^{-2} day^{-1} (average values) [35]. Thus, the presence of occupants seems to increase the concentration of airborne endotoxin considerably.

None of the other investigated parameters showed significant impact on endotoxin levels in the present study. The explanatory power of the multivariate model was the lowest among the three investigated variables, with $R^2 = 0.33$. As shown in previous studies, concentrations of endotoxin indoors depends strongly on frequency of cleaning, activity level and presence of occupants [13,17,54,56]. Furthermore, differences within homes, low frequency of vacuuming and poor ventilation habits were shown to lead to exposure to high levels of endotoxin [1,8,17,25]. Beside presence of occupants, however, these parameters were not investigated in this study, which might explain the low R^2.

Table 4
Endotoxin levels measured in tested dwellings.

	Univariate association		Multivariate association	
	% difference (95% CI)[a]	P-value	% difference (95% CI)[b]	P-value
Temperature (°C)	−9.0 (−20.7; 2.8)	0.12	−14.6 (−28.7; 29.5)	0.41
Indoor air RH (%)	1.2 (−2.5; 4.9)	0.55	3.0 (−7.0; 13.0)	0.47
Area p. person (m^2/pers.)	−3.8 (−6.8; −0.9)	0.01	−9.4 (−19.0; 0.2)	0.08
Room volume (m^3)	−0.3 (−0.9; 0.3)	0.47	1.4 (−0.6; 3.4)	0.20
Floor level				
>1st floor (n = 18)	−21.4 (−73.2; 30.3)	0.36	−11.4 (−23.7; 1.0)	0.91
ground−1st floor (n = 9)	–	–	–	–
Floor material				
Carpet (n = 4)	−40.0 (−108; 27.6)	0.15	−19.5 (−54.8; 15.8)	0.28
Wood or linol. (n = 23)	–	–	–	–
Pets				
No (n = 21)	−14.5 (−73.3; 44.3)	0.60	−28.1 (120; 64.0)	0.74
Yes (n = 6)	–	–	–	–
Year of construction				
17th century−1900 (n = 9)	−21.7 (−95.3; 52.0)	0.52	−93.5 (−262; 75.8)	0.37
1901−1960 (n = 11)	10.3 (−60.9; 81.4)	0.79	31.7 (−129; 193)	0.75
1961−present (n = 7)	–	–	–	–
AER (h^{-1})	58.4 (−67.8; 184)	0.27	63.2 (−5.4; 132)	0.17

[a] Estimated change in average endotoxin (EU m^{-2} day^{-1}) For example, an increase of the variable indoor relative humidity for 1% is interpreted as 1.2% increase of EU m^{-2} day^{-1} of endotoxin per °C.
[b] The model in the multivariate association explained 33% of the variance ($R^2 = 0.33$).

Please cite this article in press as: Spilak MP, et al., Impact of dwelling characteristics on concentrations of bacteria, fungi, endotoxin and total inflammatory potential in settled dust, Building and Environment (2015), http://dx.doi.org/10.1016/j.buildenv.2015.03.031

Results of studies investigating the impact of temperature and RH on concentrations of endotoxin are contradictory. A study performed in primary schools in Australia showed a positive correlation between endotoxin concentrations in floor dust and indoor air temperature, while inverse correlation was found with RH [50]. However, two other studies showed low effect of indoor air temperature and relative humidity on levels of endotoxin concentrations [8,21]. In our study, indoor air temperature and relative humidity showed no significant impact on endotoxin levels; however, our results are only valid in a relatively narrow range of indoor air temperature and RH.

3.4. Total inflammatory potential of settled dust

The average value of TIP in the 27 samples was 49.6 TIP m^{-2} day^{-1}, ranging from 28 to 66.9 TIP m^{-2} day^{-1}. Both uni- and multivariate analyses showed significantly lower TIP during winter season compared to spring ($p \leq 0.01$ in both associations) (Table 5). Additionally, the TIP was positively and significantly correlated with fungi concentrations (Pearson coefficient $r = 0.33$, $p = 0.04$) which is in accordance with [57]; who suggests that fungi concentration plays an important role in ability of settled dust to induce ROS.

Lower TIP was found in apartments with high $PM_{2.5}$ concentrations ($p = 0.04$) and greater room volume ($p = 0.02$). This and/or higher $PM_{2.5}$ concentrations mass might lead to varying microbial composition. Both room volume and $PM_{2.5}$ changed direction from being positively associated with TIP in univariate analysis to being negatively associated in multivariate analysis (Table 5). However, the univariate analysis was not significant for $PM_{2.5}$ concentrations or room volume.

Other parameters, such as wooden floor (as compared to carpet) and year of construction (older homes as compared to newer) did not show significant associations with TIP in multivariate analyses.

3.5. Study limitations

In this study concentrations of fungi, bacteria and endotoxin, as well as TIP, were measured in 28 dwellings. At the time of measurements, some characteristics of the dwellings, indoor climate parameters and usage habits were recorded as well. However, building characteristics were not the main focus of the study. This is reflected in the low explanatory power for the presented models, as some of the parameters shown in previous studies to have influence on the concentrations of fungi, bacteria and exotoxin were not measured in this study. This is certainly the biggest limitation of this study. The building characteristics include numerous variables which were not all captured. Furthermore, the investigated buildings were characterised by a wide variability in the characteristics of external envelopes and differences in outdoor conditions (e.g., outdoor air quality due to distance to traffic, wind pattern outdoor RH and temperature), all of which are parameters that may impact the concentrations of biological agents indoors. The study design and sample size did not allow us to implement all this diversity into our statistical models.

4. Conclusions

This study showed the size of the occupied space, together with the season, to have the biggest influence on bacteria levels in settled dust. Furthermore, bacteria levels were negatively associated with outdoor air exchange rate, indicating indoor sources dominate in contributing to the indoor bacteria levels. Relative humidity of indoor air, volume of the room and floor level also play important roles for concentrations of airborne fungi.

Aside from some dwelling characteristics, the spring season appears to have an impact on the inflammatory potential of settled dust. In conclusion, selected dwelling and building characteristics might have a predictable effect on microbial exposure.

Table 5
Total inflammatory potential of settled dust collected in 27 tested dwellings.

	Univariate association		Multivariate association	
	% difference (95% CI)[a]	P-value	% difference (95% CI)[b]	P-value
Year season				
Winter (n = 10)	−13.6 (−20.3;−6.9)	<0.01	−20.0 (−27.4; −12.6)	0.01
Spring (n = 17)	–	–	–	–
$PM_{2.5}$ (μg m^{-3})	0.2 (−1.2; 1.3)	0.98	−2.4 (−3.7; −1.0)	0.04
UFP (# cm^{-3})	0.1 (0.0; 0.1)	0.29	0.1 (−0.1; 0.1)	0.64
Temperature (°C)	−0.3 (−4.1; 4.7)	0.88	−1.7 (−6.9; 3.6)	0.57
Indoor air RH (%)	0.1 (−0.6; 0.6)	0.94	1.2 (−3.9; 6.3)	0.17
Area p. person (m^2/pers.)	0.3 (−0.2; 0.8)	0.30	0.9 (−0.1; 1.8)	0.16
Room volume (m^3)	0.1 (−0.1; 0.2)	0.30	−2.9 (−4.1; −1.6)	0.02
Floor level				
Ground floor (n = 5)	−2.3 (−14.1; 9.4)	0.70	2.5 (−74.4; 69.9)	0.75
1st floor level (n = 4)	6.7 (−5.9; 19.4)	0.31	1.8 (−9.1; 12.7)	0.76
2nd floor level (n = 6)	9.4 (−1.7; 20.6)	0.10	10.1 (−0.1; 20.4)	0.15
3rd floor level (n = 4)	4.8 (−7.8; 17.5)	0.45	−17.9 (−35.8; 0.1)	0.14
4th floor level and higher (n = 8)	–	–	–	–
Floor material				
Wood or linol. (n = 18)	2.4 (−9.1; 13.9)	0.68	22.9 (10.4; 35.4)	0.03
Parquets (n = 5)	−6.1 (−20.1; 7.8)	0.39	14.1 (−0.7; 28.9)	0.16
Carpet (n = 4)	–	–	–	–
Pets				
No (n = 21)	−1.4 (−12.9; 10.2)	0.81	−12.9 (−27.8; 2.1)	0.19
Yes (n = 6)	–	–	–	–
Year of construction				
17th century−1900 (n = 9)	7.5 (−2.3; 17.4)	0.15	9.9 (1.8; 17.9)	0.09
1901−1960 (n = 11)	11.6 (2.1; 21.1)	0.02	8.2 (−1.8; 18.1)	0.21
1961−present (n = 7)	–	–	–	–
AER (h^{-1})	0.2 (−2.3; 2.6)	0.88	−1.4 (−3.3; 0.4)	0.24

[a] Estimated change in average TIP (TIP m^{-2} day^{-1}). For example, an increase of the variable temperature for 1 °C is interpreted as 0.3% decrease of TIP m^{-2} day^{-1}.
[b] The model in the multivariate association explained 87% of the variance ($R^2 = 0.87$).

Please cite this article in press as: Spilak MP, et al., Impact of dwelling characteristics on concentrations of bacteria, fungi, endotoxin and total inflammatory potential in settled dust, Building and Environment (2015), http://dx.doi.org/10.1016/j.buildenv.2015.03.031

Conflict of interest

None of the authors have a financial relationship with a commercial entity that could have an interest in the subject of the manuscript. Each participant provided written consent before participating in the study.

Acknowledgements

The authors would like to acknowledge Professor Steffen Loft and Dr. Gabriela Karottki for recruiting volunteers for this study, and for their help with project design. This study was conducted within the Centre for Indoor Air and Health in Dwellings (CISBO). The CISBO project has been initiated and supported by the Realdania Foundation. Finally, the authors would like to thank all the occupants in the investigated dwellings.

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Please cite this article in press as: Spilak MP, et al., Impact of dwelling characteristics on concentrations of bacteria, fungi, endotoxin and total inflammatory potential in settled dust, Building and Environment (2015), http://dx.doi.org/10.1016/j.buildenv.2015.03.031